Applied and Environmental Microbiology, June 2008, p. 3583–3590 Vol. 74, No. 11
0099-2240/08/$08.00+0 doi:10.1128/AEM.02491-07


# Comparison of *Campylobacter* Populations in Wild Geese with Those in Starlings and Free-Range Poultry on the Same Farm▽

F. M. Colles,[1] K. E. Dingle,[2,3] A. J. Cody,[2] and M. C. J. Maiden[1]*

*Peter Medawar Building for Pathogen Research, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3SY, United Kingdom[1]; Nuffield Department of Clinical Laboratory Sciences, Oxford University, John Radcliffe, Hospital, Oxford OX3 9DU, United Kingdom[2]; and Department of Microbiology, Oxford Radcliffe NHS Trust, Level 6 Microbiology, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom[3]*

Received 5 November 2007/Accepted 24 March 2008

**Wild geese are a potential source of *Campylobacter* infection for humans and farm animals and have been implicated in at least two large waterborne disease outbreaks. There have been few investigations into the population biology of *Campylobacter* in geese, carriage rates are reported to vary (0 to 100%), and no genetic characterization of isolates has been performed. Fecal samples collected from wild geese in Oxfordshire, United Kingdom, were culture positive for *C. jejuni* (50.2%) and *C. coli* (0.3%). The *C. jejuni* ($n = 166$) isolates were characterized by using multilocus sequence typing and were compared with isolates collected from free-range broiler chickens and wild starlings sampled at the same location. A total of 38 STs, six clonal complexes, and 23 *flaA* SVR nucleotide STs were identified. The ST-21 and ST-45 complexes (5.4% of isolates) were the only complexes to be identified among isolates from the geese and the other bird species sampled in the same location. These clonal complexes were also identified among human disease isolates collected in the same health care region. The results indicate that large numbers of wild geese carry *Campylobacter*; however, there was limited mixing of *Campylobacter* populations among the different sources examined, and the host source could be predicted with high probability from the allelic profile of a *C. jejuni* isolate. In conclusion, genotypes of *C. jejuni* isolated from geese are highly host specific, and a comparison with isolates from Oxfordshire cases of human disease revealed that while geese cannot be excluded as a source of infection for humans and farm animals, their contribution is likely to be minor.**

*Campylobacter* is the most common bacterial cause of gastroenteritis worldwide and has an appreciable economic impact (1, 32, 40). This gram-negative organism can be readily isolated from many different environmental and animal sources and is thought to be particularly well adapted for survival in birds (10, 11, 16, 30, 37). Most human disease is sporadic and difficult to trace to the source, although outbreaks, most commonly associated with contaminated water sources or milk, are occasionally described (11, 14). A large proportion of human disease is thought to originate from the consumption of contaminated chicken meat, but the relative contributions of this and other sources are poorly defined (3, 11).

Wild geese are a potential source of *Campylobacter* infection for both humans and farm animals, and since geese are a migratory species, they are capable of transferring genotypes over large distances (16, 29, 36). Pink-footed geese have been implicated in two large outbreaks of campylobacteriosis arising from contaminated water supplies in Norway (36). Similarly, there is evidence that waterfowl such as mallard ducks are an important source of contamination for rivers (27). There have, however, been few studies investigating *Campylobacter* populations in geese, and little characterization of isolates has been undertaken. Reported carriage rates vary from 0 to 100% (2, 18, 29, 38). European starlings (*Sturnus vulgaris*) have also been identified as a source of *Campylobacter jejuni* and are common inhabitants of towns and gardens, as well as farms (28, 37).

Sequence-based typing for isolate characterization provides data that are directly comparable among host sources and are highly reproducible and amenable to population genetic analyses (7, 19). Seven-locus multilocus sequence typing (MLST) generates STs which can be grouped into clonal complexes on the basis of sharing four or more alleles in common with a central genotype. Although *Campylobacter* lineages are known to undergo frequent recombinations, lineages acquire a host signature, and previous investigations reveal that particular clonal complexes are associated with different host sources (6, 22, 25). Attribution models using allelic profiles generated by MLST have improved predictions of source and account for rare genotypes as well as those that are more broadly distributed (22). Further resolution can be obtained by nucleotide sequence determination of the *flaA* SVR sequence, permitting investigation of the short-term evolution of *Campylobacter* genotypes (6, 23, 24).

This study determined the extent to which *Campylobacter* genotypes isolated from geese were shared by another wild bird species (starlings) and by domestic chickens sampled in the same location. The goose isolates were also compared with those sampled from human disease from the same region. Lambs that were grazing pasture heavily contaminated with goose feces were examined to establish if there was any evidence of direct transfer of genotypes between the species.

* Corresponding author. Mailing address: Peter Medawar Building for Pathogen Research, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3SY, United Kingdom. Phone: 44 1865 271284. Fax: 44 1865 281275. E-mail: martin.maiden@zoology.oxford.ac.uk.

▽ Published ahead of print on 4 April 2008.

## MATERIALS AND METHODS

***Campylobacter* isolates.** A total of 331 fresh fecal samples were collected from grazing land where wild geese gathered at the Oxford University Farm in Wytham, Oxfordshire, United Kingdom. Between 40 and 84 samples were collected on five occasions from August 2002 to February 2003 and were immediately transported to the laboratory for culture. The numbers of geese exceeded 150 on each occasion and consisted of mixed species, of which the majority were Canada (*Branta canadensis*) and greylag (*Anser anser*) geese. Wild geese and starlings were present on the farm only intermittently, meaning that samples could be collected only when they were available.

A total of 954 samples from 637 individually identified wild starlings were collected at the same location between July 2002 and February 2005. Fresh fecal samples were collected and transferred to the laboratory by using charcoal transport swabs. A total of 63 flocks of free-range broiler chickens, reared at the same location on a rolling production cycle, were sampled between February and December 2003. Swabs of the anal area were collected from chickens at 56 days of age and transferred in a charcoal transport medium immediately to the laboratory for culture. A total of 84 fecal samples were collected from lambs grazing on the same pasture as that of the wild geese in October 2002 and were transferred immediately to the laboratory. The MLST and *flaA* SVR sequence data from 540 *C. jejuni* isolates collected from cases of human disease in Oxfordshire between September 2003 and September 2004 were compared with the STs identified among isolates from the geese, free-range chickens, and starlings (K. E. Dingle, N. D. McCarthy, A. J. Cody, T. E. Peto, and M. C. J. Maiden, submitted for publication).

**Bacterial culture and identification.** Samples from geese and lambs were isolated by using Preston enrichment broth (selective supplement, catalog no. SR0117; nutrient broth no. 2, catalog no. CM67; no. *Campylobacter* growth supplement, catalog SR084; and laked horse blood, catalog no. SR48 [Oxoid Ltd., Basingstoke, United Kingdom]) and cultured onto modified charcoal-cefaperazone-deoxycholate agar (mCCDA; catalog no. PO0119A; Oxoid Ltd., Basingstoke, United Kingdom). Samples from starlings were isolated by using Exeter enrichment broth (nutrient broth no. 2, catalog no. CM67; *Campylobacter* growth supplement, catalog no. SR084E; lysed defibrinated blood, catalog no. SR48 [Oxoid Ltd.]; and Exeter selective supplement, catalog no. SV59 [Mast, Bootle, United Kingdom]) and subcultured onto mCCDA. Samples from broiler chickens were cultured directly onto mCCDA.

*Campylobacter* colonies were provisionally identified by their appearance, positive catalase and oxidase reactions, and gram-negative, small curved rod morphology. The ST or allele designations resulting from the MLST were indicative of the *Campylobacter* species. Chromosomal DNA was extracted by using an IsoQuick nucleic acid extraction kit (ISC Bioexpress, Kaysville, UT), employing the protocol for rapid DNA extraction, which uses guanidine thiocyanate to chemically disrupt cells and inhibit nucleases and sodium acetate and ethanol to precipitate the nucleic acid.

**Sequence typing.** Portions of seven housekeeping genes, *aspA* (aspartase A), *glnA* (glutamine synthetase), *gltA* (citrate synthase), *glyA* (serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase), and *uncA* (ATP synthase α subunit), were sequenced. The published protocol, primers, and reaction conditions were used, except that the aspA10 primer was replaced with the aspA8 primer, 5′-CTT CCA TGT GAG GAT TTA GC-3′ (7, 26). The nucleotide extension reaction products were detected on an ABI Prism 3730 automated DNA analyzer unit and assembled using methods described previously (7). Allele numbers, STs, and clonal complexes were assigned using a *Campylobacter* MLST database (http://pubmlst.org/campylobacter/). New clonal complexes comprising at least six STs and an identifiable central genotype were distinguished by using the burst algorithm and the unweighted-pair group method using average linkages (UPGMA) cluster analysis (6, 9). The *flaA* SVR peptide was sequenced using primers and reaction conditions described previously (6, 23).

**Genetic analysis.** The pairwise Fisher statistic ($F_{ST}$) and test of significance calculations were performed using Arlequin software, version 3.0 (Arlequin, University of Geneva, Geneva, Switzerland). A sequence from each of the seven loci was concatenated using a tool on the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/), and data input files were prepared using DnaSP software, version 4.0 (33). The prediction of host origin was performed using structure software, with data input as allelic profiles (8, 22, 31). PopData and PopFlag options were used, in order that isolates external to the training set were probabilistically assigned to a host population, based upon their frequency within the host populations. The no-admixture model with lambda = 1 and independent allele frequency parameters were employed, with 1,000 burn-in cycles and 10,000 further replications for each analysis. The genetic relationships between STs isolated from the different host sources were analyzed by using ClonalFrame. A 75% consensus tree was constructed from six replicate trees, using 50,000 burn-in cycles and 100,000 further iterations (5). The model accounts for the fact that a single genetic import event may result in changes at more than one nucleotide. Concatenated nucleotide sequence data were formatted as an eXtended multi-Fasta alignment (XMFA) file, using the tool for external analysis at the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/). The genetic relationships between the *flaA* SVR types were assessed using a neighbor-joining tree, constructed using Mega software, version 3.1 (17, 34).

**Statistical analysis.** The variation in prevalence rates over time was assessed using Fisher's exact test, with associated confidence intervals based on a binomial distribution, using Stata software (StataCorp LP, TX). The distribution of clonal complexes with time was assessed using the chi-square test and associated test of significance, also using Stata. A modified version of Simpson's diversity index, together with confidence intervals (CI), was used to determine the diversity of genotypes at a given point in time while taking into account different sample sizes (13, 15). A $D$ value of 1.0 indicates that each member of a population can be distinguished from every other member, a $D$ value of 0.0 indicates that all members of a population are identical, and a $D$ value of 0.5 indicates that if one member is chosen at random from the population, there would be a 50% probability that the next strain chosen at random would be indistinguishable from the first.

## RESULTS

**Prevalence.** A total of 166 *C. jejuni* species were isolated from 331 fecal samples from geese, giving an inferred prevalence rate of 50.2% over the period of the study. On the five separate sampling occasions, the number of samples positive for *C. jejuni* were as follows: 18 August 2002, 19/40 (47.5%; CI, 31.5 to 63.9%); 11 September 2002, 37/84 (43.4%; CI, 33.2 to 55.3%); 20 September 2002, 51/82 (62.2%; CI, 50.8 to 72.7%); 3 February 2003, 32/43 (74.4%; CI, 58.8 to 86.5%); and 26 February 2003, 27/82 (32.9%; CI, 22.9 to 44.2%). The prevalence rates were significantly different ($P < 0.001$). One *C. coli* strain was isolated on 11 September 2002, equivalent to a prevalence rate of 0.3%.

The number of anal swab samples positive for *Campylobacter* among the free-range poultry was 881/975 (90.4%), with 420/975 (43.1%) samples positive for *C. jejuni* and 448/975 (45.9%) samples positive for *C. coli*. The number of fecal samples positive for *Campylobacter* among starlings was 351/954 (36.8%), with *C. jejuni* isolated from 285/954 (29.9%) samples, *C. coli* isolated from 6/954 (0.6%) samples, and *C. lari* isolated from 60/954 (6.3%) samples. *C. jejuni* was isolated from 4/84 (4.8%) fecal samples collected from lambs. No other *Campylobacter* species were isolated.

***Campylobacter* STs.** Results for the *C. jejuni* isolates are as follows. A total of 38 STs, clustering in six clonal complexes, were identified among the 166 *C. jejuni* isolates from geese (data for STs assigned to clonal complexes are given in Table 1). Seventy-five STs, clustering in 11 clonal complexes, were identified among the 285 *C. jejuni* isolates from starlings. Seventeen STs, clustering in eight clonal complexes, were identified among the 420 *C. jejuni* isolates from free-range chickens.

The ST-21 and ST-45 complexes were the only complexes present in isolates from geese (5.4% of isolates), starlings (3.2% of isolates), and free-range chickens (28.6% of isolates). In addition, isolates clustering in the ST-48 and ST-257 complexes were isolated from both the starlings (1.4% of isolates) and the free-range chickens (8.3% of isolates). Two clonal complexes, the ST-45 and ST-1332 complexes, were present in isolates from both geese and lambs.

A high proportion of the isolates from geese exhibited

TABLE 1. *C. jejuni* STs and clonal complexes isolated from test species[a]

| Clonal complex | ST | *flaA* SVR nt-peptide | Frequency (%) | | | |
|---|---|---|---|---|---|---|
| | | | Goose | Starling | Chicken | Lamb |
| 21 | 19 | 36-1 | | | 83 | |
| | | 319-15 | | | 1 | |
| | 53 | 32-1 | | | 2 | |
| | 141 | 36-1 | | | 1 | |
| | 193 | 64-1 | 1 | | | |
| | 262 | 37-1 | | | 27 | |
| | 264 | 32-1 | | | 1 | |
| | 1383 | 37-1 | | 1 | | |
| 42 | 42 | 274-42 | | 1 | | |
| 45 | 45 | 21-2 | | 2 | | |
| | | 22-1 | | 2 | | |
| | | 2-27 | | 1 | | |
| | | 15-5 | | | 4 | |
| | | 36-1 | | | 1 | |
| | 137 | 85-24 | 7 | | | 1 |
| | 706 | 85-24 | 1 | | | |
| | 998 | 21-1 | | 1 | | |
| | 1025 | 275-27 | | 1 | | |
| | 334 | 239-9 | | 1 | | |
| 48 | 38 | 41-1 | | 2 | | |
| | 475 | 105-1 | | | 21 | |
| | 813 | 41-1 | | | 2 | |
| 177 | 177 | 124-68 | | 10 | | |
| | | 403-67 | | 8 | | |
| | | 86-18 | | 7 | | |
| | | 454-24 | | 3 | | |
| | | 85-24 | | 2 | | |
| | | 322-19 | | 2 | | |
| | | 234-3 | | 1 | | |
| | | 400-43 | | 1 | | |
| | | 404-128 | | 1 | | |
| | | 460-142 | | 1 | | |
| | | U | | 10 | | |
| | 144 | 86-18 | | 2 | | |
| | | 124-68 | | 1 | | |
| | 563 | 181-94 | | 1 | | |
| | | 457-141 | | 1 | | |
| | 1004 | 447-136 | | 3 | | |
| | 1014 | 322-19 | | 1 | | |
| | 1382 | 349-67 | | 1 | | |
| | | 400-43 | | 1 | | |
| | 1482 | 322-19 | | 1 | | |
| | 1500 | 77-56 | | 1 | | |
| | 1506 | 400-43 | | 1 | | |
| | 1535 | 86-18 | | 1 | | |
| | 1381 | 432-45 | | 2 | | |
| | 1485 | 2-27 | | 1 | | |
| | 1533 | 400-43 | | 1 | | |
| | 685 | U:24 | | 1 | | |
| | 1388 | 80-48 | | 1 | | |
| | | 400-43 | | 1 | | |
| | 1394 | 461-46 | | 3 | | |
| 179 | 220 | 82-5 | | 2 | | |
| | | 68-5 | | 1 | | |
| 257 | 257 | 16-12 | | 2 | 12 | |
| 283 | 267 | 239-9 | | 1 | | |
| 460 | 1538 | 34-1 | | | 1 | |
| 573 | 573 | 8-1 | | | 82 | |
| | | 314-8 | | | 7 | |
| | | 191-33 | | | 11 | |
| | | 32-1 | | | 4 | |
| | | 67-8 | | | 1 | |
| | | 86-18 | | | 1 | |
| | 817 | 32-1 | | | 1 | |
| 574 | 574 | 8-1 | | 1 | 29 | |
| | | 66-1 | | | 1 | |
| | | 321-4 | | | 1 | |
| 661 | 814 | 191-33 | | | 111 | |
| | | 30-11 | | | 1 | |
| | 958 | 21-2 | | | 9 | |
| | | 105-1 | | | 2 | |
| | | 8-1 | | | 1 | |
| 677 | 677 | 234-3 | | 1 | | |
| | | 400-43 | | 1 | | |
| | 1024 | 234-3 | | 1 | | |
| 1534 | 234-3 | | 1 | | | |
| 682 | 682 | 62-29 | | 8 | | |
| | | U:46 | | 1 | | |
| | 1385 | 62-29 | | 1 | | |
| | 1386 | 400-43 | | 1 | | |
| | 1390 | 456-140 | | 1 | | |
| | 1392 | 81-46 | | 1 | | |
| | 1542 | 62-29 | | 2 | | |
| | 686 | 85-24 | | 10 | | |
| | | 400-43 | | 2 | | |
| | | U:24 | | 1 | | |
| | | 86-18 | | 1 | | |
| | | 45-140 | | 1 | | |
| | 1019 | U | | 1 | | |
| | 1020 | 400-43 | | 48 | | |
| | | 81-46 | | 3 | | |
| | | 124-68 | | 2 | | |
| | | 80-48 | | 1 | | |
| | | 85-24 | | 1 | | |
| | | 86-18 | | 1 | | |
| | | 181-94 | | 1 | | |
| | | 405-45 | | 1 | | |
| | | 448-137 | | 1 | | |
| | | 449-18 | | 1 | | |
| | | U | | 3 | | |
| | 1021 | 81-46 | | 3 | | |
| | | 85-24 | | 1 | | |
| | 1022 | 450-45 | | 12 | | |
| | | 451-138 | | 1 | | |
| | 1027 | 456-140 | | 7 | | |
| | | 86-18 | | 1 | | |
| | | 181-94 | | 1 | | |
| | | 349-67 | | 1 | | |
| | | U | | 3 | | |
| | 1391 | 68-5 | | 1 | | |
| | 681 | U-67 | | 1 | | |
| | 687 | U-45 | | 1 | | |
| | 818 | 404-128 | | 6 | | |
| | | 85-24 | | 1 | | |
| | | 86-18 | | 1 | | |
| | | 181-94 | | 1 | | |
| | | 186-95 | | 1 | | |
| | | 447-136 | | 1 | | |
| | 1387 | 456-140 | | 1 | | |
| | 1503 | 124-68 | | 1 | | |
| | | 450-45 | | 1 | | |
| | 1505 | 404-128 | | 1 | | |
| 692 | 1507 | 349-67 | | 1 | | |
| | 692 | 225-11 | 2 | | | |
| | | 224-35 | 1 | | | |
| | | 275-27 | 1 | | | |
| | 699 | 391-24 | 7 | | | |
| 1034 | 707 | 22-1 | 6 | | | |
| | 711 | 352-1 | 2 | | | |
| | 977 | 89-1 | 1 | | | |
| | 788 | 22-1 | 1 | | | |
| | 1033 | 199-15 | 15 | | | |
| | | 225-11 | 1 | | | |
| | 694 | 22-1 | 2 | | | |
| | 697 | 64-1 | 1 | | | |
| | 698 | 225-11 | 1 | | | |
| | 1029 | 199-15 | 1 | | | |
| 1332 | 1608 | 56-1 | 1 | | | |
| | 696 | 15-5 | 9 | | | 1 |
| 702 | 702 | 355-1 | 18 | | | |
| | 703 | 355-1 | 1 | | | |
| | 705 | 355-1 | 1 | | | |
| U | 1023 | 80-48 | | 7 | 1 | |
| | | 81-46 | | 2 | | |
| Remainder | | | 85 | 39 | 1 | 2 |

[a] The *C. jejuni* STs and clonal complexes isolated from wild geese, starlings, free-range poultry and lambs from the same farm. An additional 46 STs that could not be assigned to clonal complexes are not included, with the exception of ST-1023, isolated from both starlings and free-range chickens. Abbreviations: nt, nucleotide; U, unassigned isolate(s).

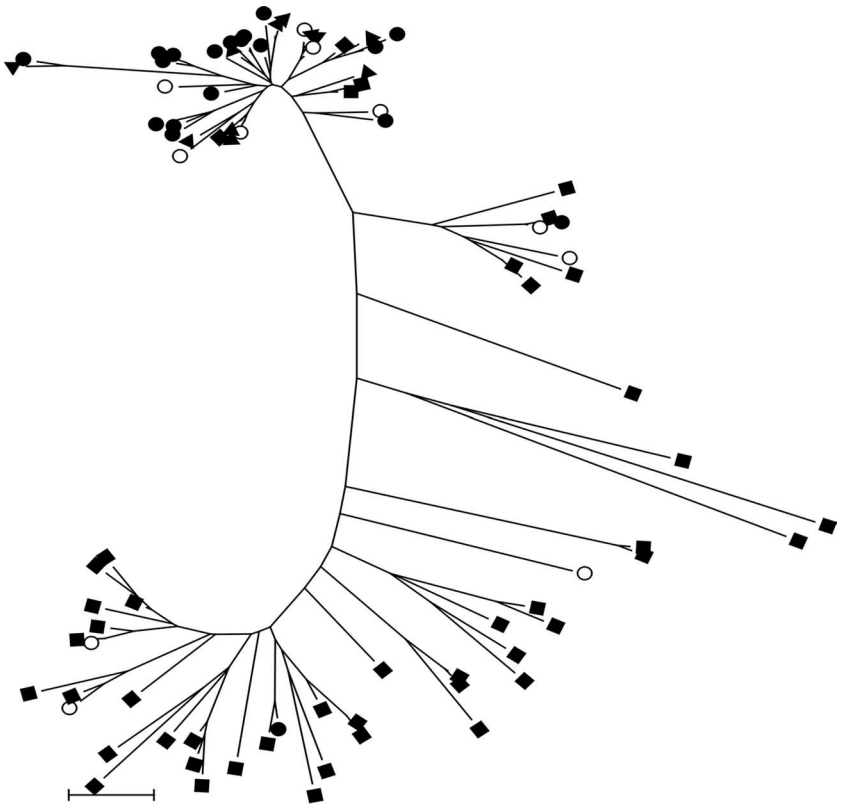

FIG. 1. A neighbor-joining tree showing the genetic relationships between the *flaA* SVR alleles isolated from geese (filled circles), starlings (filled squares), and free-range chickens (filled triangles). Open circles indicate those that are shared.

unique STs, and many of these could not be assigned to a clonal complex. No STs were shared between the geese and the other bird species. Two STs (ST-45 and ST-257) were present in isolates from both the starlings (2.5% of isolates) and the free-range chickens (4.0% of isolates), and two other STs (ST-696 and ST-137) were present in isolates from both geese and lambs. A third ST, present in isolates from lambs (ST-784), shared four of seven alleles with ST-137 but was not assigned to the same complex since it did not share four or more alleles with the central genotype.

**Antigenic typing.** Nine *flaA* SVR peptide sequences and 23 *flaA* SVR nucleotide sequences were identified among the 166 *C. jejuni* isolates from geese (Table 1 and data not shown). A total of 37 *flaA* SVR peptide sequences and 54 *flaA* SVR nucleotide sequences were identified among the 285 *C. jejuni* isolates from starlings. Ten *flaA* SVR peptide sequences and 18 *flaA* SVR nucleotide sequences were identified among the 420 *C. jejuni* isolates from free-range chickens.

Three *flaA* SVR alleles were present in isolates from both geese (10.8% of isolates) and starlings (6.7% of isolates). One *flaA* SVR allele was present in isolates from both geese (5.4% of isolates) and free-range chickens (1.0% of isolates). Seven *flaA* SVR alleles were present in isolates from both starlings (11.9% of isolates) and free-range chickens (39.0% of isolates). There was no apparent host-associated clustering of the *flaA* SVR alleles found by using a neighbor-joining tree (Fig. 1).

No identical ST-*flaA* SVR type combinations were present in isolates from geese and from the other birds tested on the farm. ST-257 was the only ST associated with the same *flaA* SVR type in isolates from starlings (0.7% of isolates) and isolates from free-range chickens (2.9% of isolates). Two of the STs present in isolates from geese and lambs had the identical *flaA* SVR types; they were ST-696, *flaA* 15; and ST-137, *flaA* 85.

**Persistence of genotypes isolated from geese over time.** Between one and five clonal complexes were identified on each

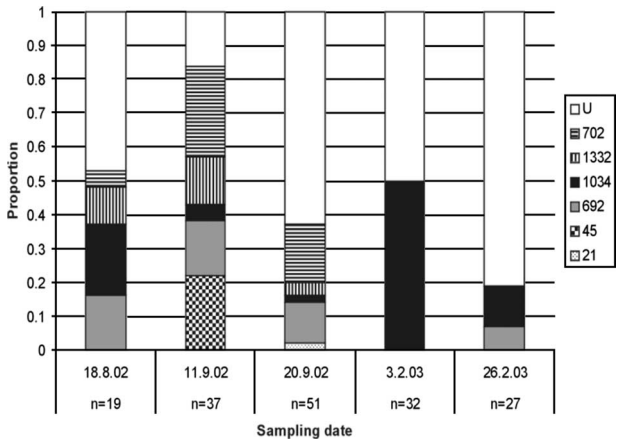


FIG. 2. Distribution of *C. jejuni* clonal complexes isolated from wild geese during the five sampling occasions in 2002 and 2003. U, unassigned isolates; number designations are ST complexes.

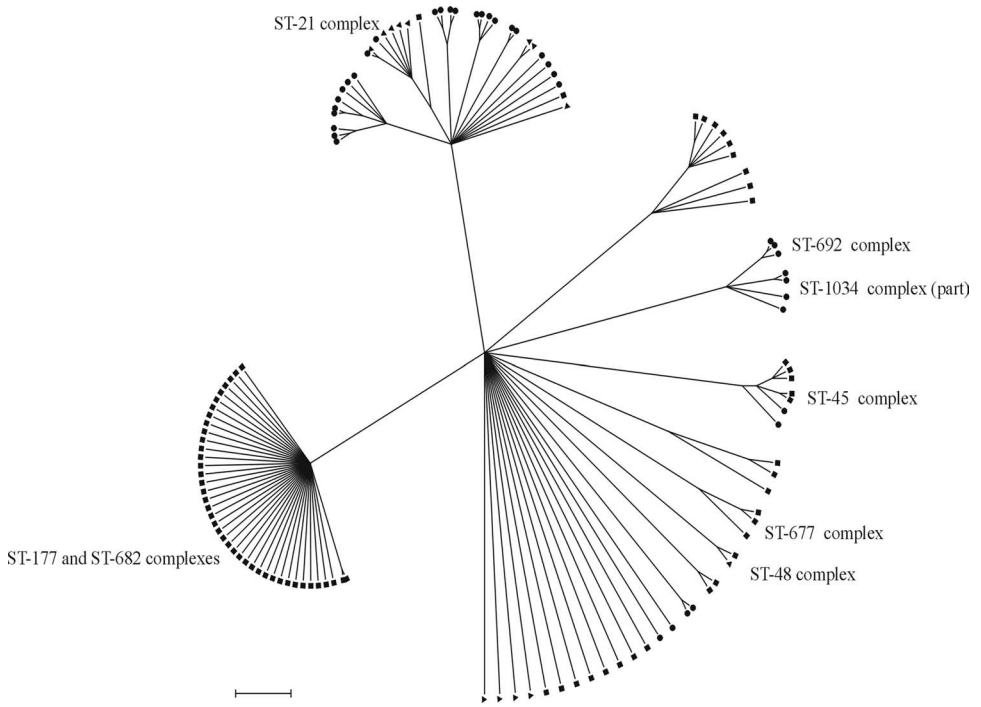


FIG. 3. A 75% consensus clonal frame tree demonstrating the genetic relationships between *C. jejuni* isolated from geese (circles), starlings (squares), and free-range broiler chickens (triangles) on the same Oxfordshire farm, using concatenated sequences. Clusters that can be related to clonal complexes are indicated.

sampling occasion, together with unassigned STs (Fig. 2). The distribution of clonal complexes over time was not random ($P < 0.001$). Only the ST-1034 complex was identified on all five occasions, although the proportion varied significantly ($P < 0.001$). The proportions of the ST-692 complex, identified on four sampling occasions, were similar each time ($P = 0.193$). Two clonal complexes, ST-21 and ST-45, were identified on only one occasion. The diversity of complexes identified among isolates collected during August and September was greater than the clonal complex diversity identified in February.

Between 10 and 16 STs were identified on each sampling occasion. The majority of STs (23/38; 60.5%) were seen on only one occasion. ST-693 was the only ST seen on four of the five sampling occasions but was associated with two *flaA* SVR alleles (data not shown). None of the STs were identified on all five occasions. The modified Simpson's index of diversity using ST data gave the highest measure of diversity for isolates collected in August 2002 ($D = 0.94$; CI, 0.91 to 0.98), followed by those collected in September 2002 (on 11 September 2002, $D = 0.88$; CI, 0.84 to 0.93%; and on 20 September 2002, $D = 0.89$; CI, 0.86 to 0.93%); those with the lowest measure of diversity were seen in February 2003 (on 3 February 2003, $D = 0.82$; CI 0.70 to 0.93%; and on 26 February 2003, $D = 0.84$; CI, 0.73 to 0.95%).

**Analysis of gene flow.** Using concatenated nucleotide sequence data, we found that the majority of goose and starling *C. jejuni* STs formed separate clusters on a ClonalFrame tree, with goose isolates more closely related to chicken isolates than the starling isolates (Fig. 3). The Gelman and Rubin statistics ranged from 1.01 to 1.50, with three of the five parameters demonstrating good convergence between the replicate trees (12). $F_{ST}$ analysis, comparing gene flow between *C. jejuni* isolated from the different host sources, gave the following results: geese and starlings, 0.604; geese and free-range chickens, 0.286; and starlings and free-range chickens, 0.617.

**Analysis of assignment probability.** With the structure software package, goose allelic profiles were correctly assigned to the goose population with an 84.2% probability, starling allelic profiles were correctly assigned to the starling population with an 80.0% probability, and chicken allelic profiles were correctly assigned to the chicken population with an 82.4% probability (Fig. 4). Eight of 14 allelic profiles that were not correctly assigned were part of the ST-21 and ST-45 clonal complexes. Another four allelic profiles clustered into clonal complexes commonly isolated from human disease (the ST-257, ST-574, ST-283, and ST-48 complexes).

**Comparison with human disease isolates.** Three clonal complexes, ST-21, ST-45, and ST-1034, were present in isolates from geese and from Oxfordshire cases of human disease (data not shown). Isolates from starlings shared seven clonal complexes with those of human cases, and isolates from free-range chickens shared eight clonal complexes with the human disease isolates (Dingle et al., submitted).

Three STs (ST-137, ST-977, and ST-1030) were present among isolates from both geese (7.2% of isolates) and human disease cases (1.7% of isolates), and six STs (ST-42, ST-45, ST-257, ST-267, ST-436, and ST-574) were present among isolates from both the starlings (4.2% of isolates) and the human disease cases (20.8% of isolates) (data not shown) (Dingle et al., submitted). Ten STs were present among isolates from both the free-range chickens (40% of isolates) and the human disease cases (27.9% of isolates).

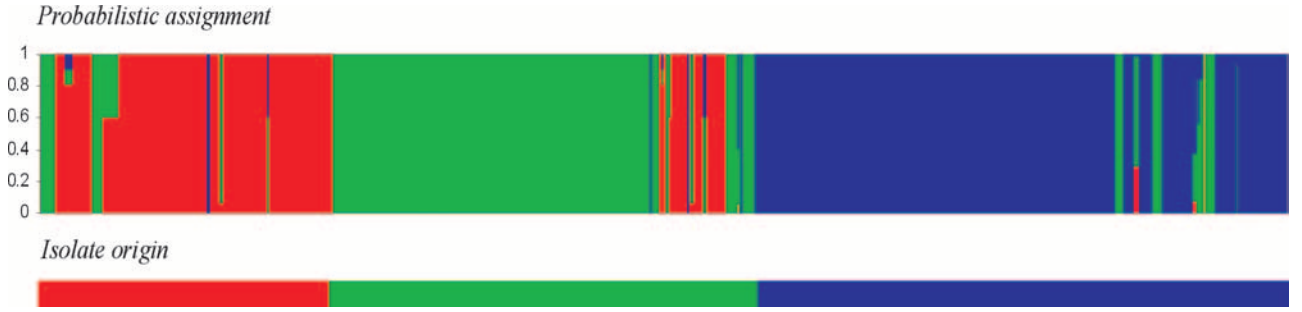


FIG. 4. Probabilistic assignment of the *C. jejuni* allelic profiles isolated from geese (red), free-range broiler chickens (green), and starlings (blue) on the same Oxfordshire farm. Each allelic profile is represented by a vertical bar, showing the estimated probability that it comes from each of the sources identified.

A total of 232 unique ST-*flaA* SVR combinations were identified among the farm and human disease isolates. None of the *C. jejuni* isolates from geese had the same ST-*flaA* SVR type combination as those isolated from human disease (Dingle et al., submitted). Five of the STs identified among isolates from starlings occurred in combination with the same *flaA* SVR type (3.5% of isolates) as those isolated from cases of human disease (11.6% of isolates). Nine of the STs identified among isolates from free-range chickens (70.5% of isolates) occurred in combination with the same *flaA* SVR type as those isolated from cases of human disease (18.4% of isolates).

## DISCUSSION

Detailed population analyses of the *Campylobacter* strains carried by geese is necessary to determine their potential for causing human disease and the extent to which they may contaminate water supplies. The 50.2% prevalence rate, inferred from the percentage of fecal samples positive for *C. jejuni*, among wild geese reported in this study was higher than the carriage rates reported for wild greylag and Canada geese in the United States and Scandinavia (0 to 24%) but lower than the carriage rate recorded among domestic geese in Turkey (100%) (2, 18, 29, 38). The differences may be partly explained by the different sampling regimens used in the studies, for example, enrichment methods and intestinal samples giving a higher isolation rate than direct culture and fecal samples, and also by sampling wild geese versus farmed geese (35). The prevalence rate of *C. jejuni* varied significantly with the different sampling occasions, irrespective of the season, indicating that individual geese within a flock were not colonized independently of one another. Other factors such as the changing dynamics of migrating flocks or fluctuations in agricultural run-off may be important (27).

A strategy whereby single colonies were picked from a large number of fecal samples, rather than multiple colonies picked from a small number of fecal samples, was adopted to maximize the number of independent isolations and thus capture the diversity and rarer *Campylobacter* genotypes. The MLST data demonstrated high genetic diversity among *C. jejuni* isolates from geese. In common with other studies of wildlife, a large proportion of the STs remained unassigned (10). Interrogation of the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/) revealed few isolates related to those obtained from geese and suggests that the ST-692, ST-702, ST-1034 and ST-1332 complexes may be associated with this host species. A ClonalFrame analysis indicated that the goose genotypes were not monophyletic and shared common ancestors with genotypes isolated from chickens and starlings. Full resolution of the ST-682 and ST-177 complexes may require sequence data from additional loci.

Greater diversity in *C. jejuni* genotypes was seen in late summer/early autumn ($D = 0.88$ to 0.94) than in the winter ($D = 0.82$ to 0.84); however, the CIs indicate that the differences were small. There was a predominance of a single ST during the winter months and a more even distribution of STs in the summer months. Although the clustering of clonal complexes was uneven over time, the differing prevalence rates could not be linked with particular STs; rather, the majority (57.9%) were sporadic and were isolated on only one sampling occasion.

There was little evidence of cross-contamination of *C. jejuni* genotypes between geese, starlings, and free-range chickens on the same farm. Only one of the 232 ST-*flaA* SVR combinations identified among the farm and human disease case isolates in this study was identified among isolates from the starlings and free-range chickens, and this occurred at low frequency in both host sources. The large significant $F_{ST}$ values, high probabilistic assignment results, and separate clusters on a ClonalFrame tree were inconsistent with a single population of *C. jejuni* on the farm and gave further evidence that the mixing of *C. jejuni* populations among the different host species tested was limited. The prediction of host origin based on the allelic profile was higher than that seen by McCarthy et al., who used isolates from cattle, sheep, and chickens, suggesting that *Campylobacter* genotypes may circulate more commonly among different farm animal species than among different wild bird species (22). All of the time frames for the different host species compared in this study overlap and were identified within a 4-year period; thus, sampling effects are likely to be minimal, given results from a previous study comparing *Campylobacter* populations over decades and between continents, which revealed a greater host association than temporal or space association (22, 38). Similarly, other sampling effects, such as those resulting from the use of isolates from processed carcasses versus those from live birds, were small in comparison to host effects, and thus any effects introduced by different culture techniques are also likely to be small (22). Samples

from geese, lambs, and starlings required enrichment, and all were cultured onto mCCDA; the main difference was that campylobacters grown in Preston broth were subject to cyclohexamide, an inhibitor of protein synthesis in eukaryotic rather than prokaryotic organisms.

The *flaA* SVR types were widely distributed among isolates and were shared among STs and clonal complexes and also host sources, demonstrated on the neighbor-joining tree. Use of the *flaA* SVR sequence alone would be an unreliable indictor of the relationships among isolates; however, combined with MLST, the *flaA* type enhanced discrimination (6). Despite the fact that flagellin is an important colonization factor promoting interaction between host and pathogen, it appeared to have less host specificity than the housekeeping genes used for MLST (20, 39, 41). The reasons for this are not clear.

Very few *C. jejuni* species were isolated from the lambs grazing the pasture that was heavily contaminated by goose feces, despite the use of enrichment culture. Results from another study indicate that colonized lambs do not always shed significant numbers of *Campylobacter* in feces, or the bacteria may be shed intermittently (35). No ruminant-associated genotypes were isolated; rather, two ST-*fla* SVR types identical to those isolated from geese, together with a third closely related ST, suggested that the lambs may have been colonized, if only briefly, by campylobacters from geese. However, the inference that can be made is limited due to the low rates of isolation (4, 6, 21). Occasional isolates from other farm animals, wild birds, and environmental waters were recorded among the goose-associated clonal complexes on the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/), but it seems likely that such strains are less successful in the alternative hosts.

There was no evidence that *C. jejuni* strains characterized by the ST and the *flaA* SVR type observed with geese were associated with cases of human disease sampled in the same region (Dingle et al., submitted). Similarly, only three of the STs identified from among the goose isolates were observed in the current listing of 1,955 isolates from human disease worldwide on the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/), and these occurred at low frequency (0.7% of isolates). Two of the STs were from the ST-21 and ST-45 complexes, which are large and diverse, suggesting they may be able to survive and spread in multiple hosts (4, 6). It is not possible from these data to ascertain whether geese are a true reservoir of the ST-21 and ST-45 genotypes or whether the geese acquire the genotypes indirectly from humans or farm animals, for example, from agricultural runoff (27). The possibility of *C. jejuni* strains colonizing geese, leading to human disease and waterborne outbreaks, cannot be excluded, but it is likely to be a rare event.

Although five isolates from starlings were characterized by an ST and a *flaA* SVR type in common with the Oxfordshire human disease isolates, all were identified infrequently from both host types (K. E. Dingle et al., manuscript submitted). In contrast to the isolates from geese and starlings, 70% of the *C. jejuni* isolates from free-range chickens were indistinguishable by ST and the *flaA* SVR type from Oxfordshire human disease isolates. The results indicate that chickens are a far more likely source of human disease than geese and starlings, most likely reflecting different exposure rates. The fact that *C. jejuni* genotypes present in a small free-range chicken production unit can be isolated from disease cases of humans exposed to nationally produced and distributed meat suggests that particular genotypes can be linked to chicken production.

In conclusion, geese have the potential to be a reservoir of *C. jejuni*, with an average of 50.2% positive fecal samples in this study. There was high genetic diversity among the *C. jejuni* isolates, with evidence of host association, although this was not restricted to a single genetic lineage. There was no evidence for cross-contamination of genotypes identifiable by ST and *flaA* SVR between geese and the starlings and free-range poultry tested on the same farm or an association with human disease in the same region. The *C. jejuni* strains colonizing geese do not appear to represent a high proportion of those sampled from human disease cases, although the possibility that such strains could cause human disease or waterborne outbreaks on rare occasions cannot be completely dismissed.

## ACKNOWLEDGMENTS

We thank Ruth Layton and the Food Animal Initiative, Oxfordshire, United Kingdom, for providing access to the chicken flocks and wild birds, Julian Howe for assistance with sampling, Noel McCarthy for statistical advice, and the Oxford University Zoology Sequencing Facility.

This work and the maintenance of the *Campylobacter* MLST database (http://pubmlst.org/campylobacter/) were funded by the United Kingdom Department for Environment, Food and Rural Affairs, contract no. OZ0604.

## REFERENCES

1. **Allos, B.** 2001. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis. **32:**1201–1206.
2. **Aydin, F., H. I. Atabay, and M. Akan.** 2001. The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). J. Appl. Microbiol. **90:**637–642.
3. **Baker, M., N. Wilson, R. Ikram, S. Chambers, P. Shoemack, and G. Cook.** 2006. Regulation of chicken contamination is urgently needed to control New Zealand's serious campylobacteriosis epidemic. N. Z. Med. J. **119:** U2264.
4. **Colles, F. M., K. Jones, R. M. Harding, and M. C. Maiden.** 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl. Environ. Microbiol. **69:**7409–7413.
5. **Didelot, X., and D. Falush.** 2007. Inference of bacterial microevolution using multilocus sequence data. Genetics **175:**1251–1266.
6. **Dingle, K. E., F. M. Colles, R. Ure, J. Wagenaar, B. Duim, F. J. Bolton, A. J. Fox, D. R. A. Wareing, and M. C. J. Maiden.** 2002. Molecular characterisation of *Campylobacter jejuni* clones: a rational basis for epidemiological investigations. Emerg. Infect. Dis. **8:**949–955.
7. **Dingle, K. E., F. M. Colles, D. R. A. Wareing, R. Ure, A. J. Fox, F. J. Bolton, H. J. Bootsma, R. J. L. Willems, R. Urwin, and M. C. J. Maiden.** 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39:**14–23.
8. **Falush, D., M. Stephens, and J. K. Pritchard.** 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164:**1567–1587.
9. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518–1530.
10. **French, N., M. Barrigas, P. Brown, P. Ribiero, N. Williams, H. Leatherbarrow, R. Birtles, E. Bolton, P. Fearnhead, and A. Fox.** 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ. Microbiol. **7:**1116–1126.
11. **Frost, J. A.** 2001. Current epidemiological issues in human campylobacteriosis. Symp. Ser. Soc. Appl. Microbiol. **2001:**85S–95S.
12. **Gelman, A., and D. B. Rubin.** 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. **7:**457–472.
13. **Grundmann, H., S. Hori, and G. Tanner.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192.
14. **Hänninen, M. L., H. Haajanen, T. Pummi, K. Wermundsen, M. L. Katila, H. Sarkkinen, I. Miettinen, and H. Rautelin.** 2003. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organ-

isms in three waterborne outbreaks in Finland. Appl. Environ. Microbiol. **69:**1391–1396.
15. **Hunter, P. R.** 1990. Reproducibility and indices of discriminatory power of microbial typing methods. J. Clin. Microbiol. **28:**1903–1905.
16. **Jones, K.** 2001. Campylobacters in water, sewage and the environment. J. Appl. Microbiol. **30:**68S–79S.
17. **Kumar, S., K. Tamura, and M. Nei.** 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. **5:**150–163.
18. **Lillehaug, A., C. Monceyron Jonassen, B. Bergsjo, M. Hofshagen, J. Tharaldsen, L. L. Nesse, and K. Handeland.** 2005. Screening of feral pigeon (*Colomba livia*), mallard (*Anas platyrhynchos*) and graylag goose (*Anser anser*) populations for *Campylobacter* spp., *Salmonella* spp., avian influenza virus and avian paramyxovirus. Acta Vet. Scand. **46:**193–202.
19. **Maiden, M. C.** 2006. Multilocus sequence typing of bacteria. Annu. Rev. Microbiol. **60:**561–588.
20. **Malik-Kale, P., B. H. Raphael, C. T. Parker, L. A. Joens, J. D. Klena, B. Quinones, A. M. Keech, and M. E. Konkel.** 2007. Characterization of genetically matched isolates of *Campylobacter jejuni* reveals that mutations in genes involved in flagellar biosynthesis alter the organism's virulence potential. Appl. Environ. Microbiol. **73:**3123–3136.
21. **Manning, G., C. G. Dowson, M. C. Bagnall, I. H. Ahmed, M. West, and D. G. Newell.** 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl. Environ. Microbiol. **69:**6370–6379.
22. **McCarthy, N. D., F. M. Colles, K. E. Dingle, M. C. Bagnall, G. Manning, M. C. Maiden, and D. Falush.** 2007. Host-associated genetic import in *Campylobacter jejuni*. Emerg. Infect. Dis. **13:**267–272.
23. **Meinersmann, R. J., L. O. Helsel, P. I. Fields, and K. L. Hiett.** 1997. Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. J. Clin. Microbiol. **35:**2810–2814.
24. **Meinersmann, R. J., R. W. Phillips, K. L. Hiett, and P. Fedorka-Cray.** 2005. Differentiation of *Campylobacter* populations as demonstrated by flagellin short variable region sequences. Appl. Environ. Microbiol. **71:**6368–6374.
25. **Miller, W. G., M. D. Englen, S. Kathariou, I. V. Wesley, G. Wang, L. Pittenger-Alley, R. M. Siletz, W. Muraoka, P. J. Fedorka-Cray, and R. E. Mandrell.** 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. Microbiology **152:**245–255.
26. **Miller, W. G., S. L. W. On, G. Wang, S. Fontanoz, A. J. Lastovica, and R. E. Mandrell.** 2005. Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J. Clin. Microbiol. **43:**2315–2329.
27. **Obiri-Danso, K., and K. Jones.** 1999. Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England. J. Appl. Microbiol. **87:**822–832.
28. **Odermatt, P., S. Gautsch, D. Rechsteiner, R. Ewald, D. Haag-Wackernagel, R. Muhlemann, and M. Tanner.** 1998. Swarms of starlings in Basel: a natural phenomenon, a nuisance or a health risk? Gesundheitswesen. **60:**749–754. (In German.)
29. **Pacha, R. E., G. W. Clark, E. A. Williams, and A. M. Carter.** 1988. Migratory birds of central Washington as reservoirs of *Campylobacter jejuni*. Can. J. Microbiol. **34:**80–82.
30. **Petersen, L., E. M. Nielsen, J. Engberg, S. L. On, and H. H. Dietz.** 2001. Comparison of genotypes and serotypes of *Campylobacter jejuni* isolated from Danish wild mammals and birds and from broiler flocks and humans. Appl. Environ. Microbiol. **67:**3115–3121.
31. **Pritchard, J. K., M. Stephens, and P. Donnelly.** 2000. Inference of population structure using multilocus genotype data. Genetics **155:**945–959.
32. **Roberts, J. A., P. Cumberland, P. N. Sockett, J. Wheeler, L. C. Rodrigues, D. Sethi, and P. J. Roderick.** 2003. The study of infectious intestinal disease in England: socio-economic impact. Epidemiol. Infect. **130:**1–11.
33. **Rozas, J., J. C. Sanchez-DelBarrio, X. Messeguer, and R. Rozas.** 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics **19:**2496–2497.
34. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
35. **Stanley, K. N., J. S. Wallace, J. E. Currie, P. J. Diggle, and K. Jones.** 1998. Seasonal variation of thermophilic campylobacters in lambs at slaughter. J. Appl. Microbiol. **84:**1111–1116.
36. **Varslot, M., J. Resell, and I. G. Fostad.** 1996. Water-borne campylobacter infection–probably caused by pink-footed geese. Two outbreaks in Nord-Trondelag, Stjortdal in 1994 and Verdal in 1995. Tidsskr. Nor. Laegeforen. **116:**3366–3369. (In Norwegian.)
37. **Waldenström, J., T. Broman, I. Carlsson, D. Hasselquist, R. P. Achterberg, J. A. Wagenaar, and B. Olsen.** 2002. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Appl. Environ. Microbiol. **68:**5911–5917.
38. **Waldenstrom, J., S. L. On, R. Ottvall, D. Hasselquist, and B. Olsen.** 2007. Species diversity of campylobacteria in a wild bird community in Sweden. J. Appl. Microbiol. **102:**424–432.
39. **Wassenaar, T. M., B. A. van der Zeijst, R. Ayling, and D. G. Newell.** 1993. Colonization of chicks by motility mutants of *Campylobacter jejuni* demonstrates the importance of flagellin A expression. J. Gen. Microbiol. **139:**1171–1175.
40. **Withington, S. G., and S. T. Chambers.** 1997. The cost of campylobacteriosis in New Zealand in 1995. N. Z. Med. J. **110:**222–224.
41. **Yao, R., D. H. Burr, P. Doig, T. J. Trust, H. Niu, and P. Guerry.** 1994. Isolation of motile and non-motile insertional mutants of *Campylobacter jejuni*: the role of motility in adherence and invasion of eukaryotic cells. Mol. Microbiol. **14:**883–893.